Is the timing of caloric intake associated with variation in diet-induced thermogenesis and in the metabolic pattern? A randomized cross-over study

19 Is the Timing of Caloric Intake Associated with Variations in Energy Expenditure and Metabolic
20 Pattern? A Randomized Crossover Study.

21
22 Simona Bo[1*], Maurizio Fadda[2], Giovannino Castiglione[3], Antonella De Francesco[2],
23 Federica Fedele[1], Alice Gambino[1], Marilena Parasiliti Collazzo[1], Chiara Ponzo[1], Maria Adele Bo[1], Giulio Menato[4], Egidio
24 Ghigo[1], Mauro Maccario[1], Fabio Broglio[1]
25
26 [1]Department of Medical Sciences, Turin, Italy, University of Turin
27 [2]Unit of Clinical Nutrition, Città della Salute e della Scienza Hospital of Turin, Turin
28 [3]Unit of Clinical Epidemiology, CPO, Città della Salute e della Scienza Hosp
29 [4]Clinical Biochemistry Laboratory, Città della Salute e della Scienza, Turin, Italy
30
31 *Corresponding author: Simona Bo, Department of Medical Sciences, University of Tu
32 10126 Turin, Italy; Telephone +(39)(011)6336836, Email: simona.bo@unito.it, Fax (39)(011)6335

33
34
35 Running title: Timing of caloric intake, thermogenesis and metabolism
36 Word Count: abstract 295, text 3885
37 Conflicts of interest: The authors report no conflict of interest.
38 Financial support: This study was supported by Ministry of Education, University and
39 of Italy (60% x 2014)
40
41
42

Abstract

Background/Objectives: Individual differences in the postprandial glycemic response are generally reported to be higher in the morning than in the evening, although contrasting evidence exists. Studies to date have not controlled adequately for preceding fasting, exercise, sleep, and dietary factors. The present study addressed whether differences in postprandial metabolic responses to identical meals between morning and evening are observed when these factors are controlled.

Subjects/Methods: Twenty-three healthy volunteers (age range 20–35 years, BMI 20–28 kg/m²) were enrolled and randomized over two visits. They only differed in the time of the same standardized meal, and this was given 7 days after, in the observation period. After an overnight fast, basal calorimetry was performed; a 60-min calorimetry was started 120 min after the beginning of the meal. Blood samples were drawn every 30 min for 180 min. General linear models (GLM), adjusted for order of the two visits, were used to evaluate the effect of time, i.e. the difference of morning minus evening values (after evaluating that fasting glucose, insulin, and resting energy expenditure (REE) did not differ between visits). Postprandial glucose, insulin, and free fatty acid concentrations were assessed as incremental areas under the curve. Diet-induced thermogenesis (DIT) was calculated as the increase in energy expenditure above REE.

Results: Postprandial glucose (mean 4.6; 95% CI 4.5, 4.8 mmol/l; $p = 0.016$) and insulin (mean 39; 95% CI 32, 48 mU/l; $p = 0.001$) were significantly higher after the morning meal than after the evening meal. Glucose (mean 1.3; 95% CI 0.8, 1.8 mmol/l; $p < 0.001$) and insulin (mean 0.7; 95% CI 0.4, 1.0 mU/l; $p < 0.001$) were significantly increased after the morning meal. Delay and overall larger increases in glucose and insulin were found after the evening meals. Conclusions: The same meal consumed in the morning, as compared to the evening, induced increased glycemic and insulinemic responses, suggesting that the evening may be a vulnerable time of the day for metabolic patterns typical of healthy individuals. This finding should probably be considered when nutritional recommendations are given.

ClinicalTrials.gov NCT01823380

Introduction

An increasing number of studies have shown that the timing of food intake influences energy balance, risk of weight gain, and the development of obesity. Total daily caloric intake, macronutrient composition and eating frequency have been identified as important factors influencing energy expenditure [1].

Early insulin secretion after glucose ingestion is significantly higher in the morning than in the evening, leading to a more rapid glucose clearance from the blood [2], whereas gastric emptying is slower [3] and insulin sensitivity lower [4]. Circadian variations in the concentrations of hormones and peptides such as ghrelin, leptin, and adiponectin, as well as genes that are components of the circadian clock, have been implicated in the daily modification of metabolic pathways [1]. While gastric emptying seems to be more rapid in the morning than in the evening [5], an increased efficacy of dietary carbohydrates absorption has been reported in the evening [6]. Such circadian variations in digestive processes may contribute to these observed differences in postprandial glucose metabolism [7].

A few studies evaluated the circadian variation of the energy balance component known as diet-induced thermogenesis (DIT), also called the thermic effect of food. DIT, which is defined as the increase in resting metabolic rate (RMR) after the ingestion of a meal, is the least studied component of energy expenditure, although it has been reported to be diminished in the elderly and in persons with obesity [8]. DIT is significantly higher after the consumption of a meal than after the consumption of a snack at night [9], and a reduced thermogenic response may contribute to the positive energy balance [10] observed in night workers [11]. Furthermore, habitual nighttime eating or snacking have been associated with a potentially promoting weight gain [12].

However, data in the literature show contrasting results [9, 12] with differences in the level of exercise, hours of sleeping, and even in the duration of fasting or caloric density, which could be explained by the low number of the subjects recruited [13]. Accordingly, the ideal setting to study diurnal variations in exercise metabolism or postprandial RMR is to measure these parameters under the same conditions at different times. The aim of our study was to compare the effects of a meal on the metabolic

responses to identical protein, carbohydrate and fat meals consumed in the morning (8:00 in the evening (20:00) by healthy volunteers after standardizing diet, physical activity and resting.

Subjects and Methods

Recruitment of participants

Twenty healthy (ten males and ten females) volunteers were enrolled among students and graduates from the Department of Medicine (Table 1).

Inclusion criteria: age 20–35 years, body mass 46–62 kg, body mass index (BMI) 18.5–24.9 kg/m², moderate exercise level (<3 times/week), smoking <10 cigarettes/day. Exclusion criteria: acute or chronic diseases, drugs or supplementation, dietary restrictions or specific food dislikes, unwillingness to give a written informed consent.

Design

This was a randomized controlled trial.

Outcomes

The primary outcomes were changes in RMR after the meals compared to changes in RMR after the evening meal. The secondary outcomes were changes in circulating concentrations of glucose, free fatty acids (FFA) and triglycerides during the morning and evening consumption.

Intervention

Participants were randomly allocated to receive a standard meal (SM) on the first day and the week after the standard meal (SM), or vice versa. On the first day of the meal (respectively 8:00 am and 20:00 pm), participants received the same standard (without protein supplementation) meal, and then asked to stay in the laboratory for 6 h. Drinking coffee, alcohol, and tea were not allowed during the

permitted. The week preceding the test, participants were instructed to avoid alcohol and to refrain from heavy physical activity. A 24-hour urine collection was collected the day before determined urinary nitrogen excretion.

The standard meal offered 100g white bread, 100g ham, 50g cheese, 125g yo 25g protein supplement (Resource Instant Protein, Nestlé, Switzerland). The energ meal was 30% protein, 31% carbohydrate, 39% fat. The participants consume each meal in 30 min. Participants take every day a car to the laboratory at 7:00 am (or pm, according to randomization), the upper limb vein access and to the insertion of indwelling catheter into an antecubital vein of the forearm, subsequen 500 ml of saline solution until the end of the testing session. The first blood the blood samples have been withdrawn from an extension line tubing.

A 30-min basal calorimetric examination was performed. Participants remained in a supine position awake and motionless for the whole period preceding the meal, when they could eat and were allowed to. At 8:00 am (or pm), the participants underwent the supine position for 90 min, followed by a second calorimetric evaluation to obtain a compliance to the experiment, the second calorimetric measurement (from 120 to 180 min after the beginning of the meal) was previously performed in a pilot study to evaluate the tolerance to the calorimetric device. It was found that maintaining immobility while more than 1 hour consecutive.

Blood samples were drawn every 30 min during the first calorimetric session (at the end of the se (post-prandial). Figure 1). Time 0 was after the first calorimetry and before the meal. T and 0 were referred to the time interval beginning. The same time schedule was adopted in the case of the morning meal (at 8:00 am). an second test was carried out after 7 days from the first.

Sample size

A sample size of 20 subject-first (10 in the and 10 weight group) was
to test 0.66 effect size with 80% and a two-tailed value = 0.05.

Randomization

The random sequence (morning/evening) was computer-generated, using blocks
different lengths in random order.

Measurements

The Minnesota Leisure Time Physical Activity Questionnaire [16], previously used in an Italian cohort
[17], was performed by all the participants together with a 3-day food record, which consisted of
written food [18]. Subjects were instructed to record every ingredient of the
week days and one Saturday-Sunday. The 3-day food record data were loaded on the Win Food
(Medimatica, Colonnella, Teramo, Italy), and the mean nutritional values
Body weight and height were measured with subjects wearing light clothes and no shoes, using a medical scale column
(SECA, Hamburg, Germany) to the nearest 0.1 kg and 0.1 cm, respectively. Waist circumference was
measured by a plastic tape meter at the top of the iliac crests. Fat mass and fat-free mass were norm
were determined by a bioelectrical impedance analysis (BIA) device (BIA 101, Akern
Italy). Indirect calorimetry by a ventilated hood system (Delta Trac, Datex Instruments, Helsinki
measure the rate of energy expenditure, by measuring the inspired O_2 and exp
and carbon dioxide (CO_2) production. Detailed methodology can be found elsewhere [19]. Indirect calorimetry reflects nutrient metabolism and is one of the reference
tools for reliable measurements, in vivo, in humans [20]. The accuracy of the device has been tested in several
studies [21]. Before measurements, the instrument was calibrated according to the manufacturer's instructions. A transparent canopy was placed over
the subject's head and face and carefully checked to prevent air leaks. After a 30-min period of resting metabolic rate, gas sample
were continuously analyzed by paramagnetic and infrared gas analyzers for O_2 and CO_2 concentrations, respectively. Ambient
During the calorimetric exams, room temperature was kept between 20 and 22 °C. The exams were performed in

163 room with a temperature of 22–25°C. The energy expenditure was calculated from the
164 beginning of the meal, because it has been reported that the DIT respo
165 sufficient precision when the movements of the monitored wrist mirror
166 immobility [15].
167 Blood samples were immediately centrifuged and aliquots of plasma we
168 analysis. The following determinations were performed: glucose, FFA, triglycerides. Serum glucose was
169 measured by enzymatic assay (Merlin Diagnostics, Florence, Italy); serum
170 determined by immunoradiometric assay (Beckman Coulter, Immunotech, Prague, Czech
171 coefficients of variation were 3.2–9.1% and inter-assay coefficients of variation 4.0–8.7%). FFA concentrations were
172 measured by a fluorometric assay (Wako NEFA, Wako Chemicals, Neuss, Germany). Triglycerides were assa
173 enzymatic colorimetric method (Hitachi, Roche Diagnostics, Mannheim, Germany). Urinary nitrogen
174 excretion was determined from the 24-h collection of the day before each test. Tot
175 was assessed by a kinetic assay (Hitachi, Roche Diagnostics, Mannhei
176 Definitions
177 The physical activity level was calculated as the product of the duratio
178 hours/week), weighted by an estimate of the metabolic equivalent of th
179 activities performed.
180 Both basal and mean RMR were calculated according to [20]. RMR was calculated [a
181 in relation to fat free mass (FFM) and was as: RMR/FFM and expressed as
182 DIT was considered as the difference between the mean RMR and the basal RMR (mean RMR
183 basal RMR).
184 The Respiratory Quotient (RQ) was the ratio between VCO_2 and VO_2: VCO_2/VO_2.
185 Glucose and fat oxidation were calculated according to the Frayn formula [21]:
186 Carbohydrate oxidation (g/min) = $4.55 \times VCO_2$ (l/min) – $3.21 \times VO_2$ (l/min) – $2.87 \times N$ (g/min)

187 Fat oxidation (g/min) = 1.67 VO2 (l/min) - 1.92 VCO2 (l/min) - 1.92 N (g/min)

188 VO2 = oxygen consumption, VCO2 = carbon dioxide production; N = urinary nitrogen exc

189 Rate of N, an index of protein oxidation, was assumed to be constant during the calorimetry.

190 Area under the curve (AUC) for glucose, insulin, FFA and triglycerides were calcu

191 trapezoidal model [22].

192 We defined as Delta the difference between each variable at fasting and after the

193 Delta = variable value after the meal - variable value at fasting

194 In the case of the calorimetric variables, the values at fasting corresponded to

195 calorimetry; delta RMR therefore corresponded to the difference between RMR at fasting

196 fasting corresponded to the values at time 0.

197 Blinding

198 Due to the nature of the intervention, blinding participants and health

199 laboratory personnel to the group assignments was not possible. Data analysts were

200 Ethics

201 The study was approved by the Ethics Committee of the Società della Salute e della Scienza of T

202 procedures conformed to the principles of the Helsinki Declaration of 1975, as revised in

203 consent to take part to the study.

204 Statistical methods

205 Clinical and laboratory variables were expressed as means ± SD. Data for glucose, insulin and

206 values were logarithmically transformed in order to obtain a Gaussian distribution before

207 paired data was applied with the subject serving as the variable being tested at evening

208 With-subject changes in the variables in the morning and the evening from the calorie consump

209 analysis to estimate the effect on the difference between the delta and the evening

210 delta

211 Morning effect = morning value − evening value

212 In the case of AUCs, the morning effect was the difference between mor
213 evening AUC for the variable.
214 General linear models (GLM) with patients as random effects were per
215 possible period and carryover effects and to estimate morning and evening means and 95 %
216 confidence intervals (95% CI). In order to make easier the interpretation of the
217 adjusted estimates of triglycerides and insulin AUCs were expressed b
218 In an explorative analysis GLM were analyzed for glucose, insulin, FFA and
219 values at single time points.
220 The repeated measures from 0 to 180 minutes of glucose, insulin, FFA
221 reported in tables and as means and 95 % confidence intervals at time 0
222 Statistical analyses were performed using SAS/STAT 13.1 (SAS Institute, Cary, Texas).
223
224 Results
225 Mean age, weight, height, body mass index (BMI), and waist circumfer
226 years, 67.3 ± 12.5 kg, 1.70 ± 0.1 m, 23.8 ± 3.4 kg/m^2, and 82 ± 10 cm, respectively. Fat mass an
227 determined by bioelectrical impedance analyses were 14.5 ± 6.0 kg and
228 Participants were moderately active: their median METs h/week was 4
229 low fiber diet (total kcal 1989.9 ± 523.0; fat 39.9 ± 15.7 % total kcal; saturate
230 monounsaturated fatty acids 14.9 ± 4.2 % total kcal; carbohydrates 46.6
231 There were no meaningful differences between the two periods (first morning
232 first for anthropometrics RMR values (Table 1 SI, Supplementary Information).
233 In Table 1 the morning and evening calorimetric variables that were measured and
234 reported. Fasting was slightly lower in the morning. After meal RMR and DIT values were significantly higher

235 after the morning and after the evening meal. Fasting and mean AUC values at morning were significantly higher th
236 corresponding RQ. Similarly, fasting and mean AUC values of CHO oxidation were significantly higher a
237 fasting and mean AUC values of fat oxidation were significantly lower in the morning than in the evening. Morning va
238 Period and sequence effects were tested using GLM and restricted to statistically significant
239 variables. These crude mean values reflect the effects of morning and evening meals, but differences may
240 adjusted effects were chosen for the analysis of RMR, via the difference in DIT, to indicate the greater
241 DIT increase after the morning meal than after the evening meal. Additionally, differences in the area under the
242 RQ values, as well as the differences between morning and evening meals in glucose, insulin and FFA concentrations
243 were negative, indicating a greater effect of the evening meal on these variables than the morning
244 meal.
245 Adjusted estimated means of the effects tested by GLM showed significantly lower glucose at
246 time 60, 90, 120, 150 values of insulin at time 90, 120, 150, 180, 210, 240 and lower values of triglycerides at time 60, 90, 120, 150, 180,
247 and lower values of triglycerides at time 60, 90, 120, 150, 180, 210, 240 (Tables 2, 3 and 4 for more information).
248 Basal values of glucose, insulin, FFA and triglycerides (Figures 1, 2, 3 and 4) differ f
249 (panels A) and values according to the different time points of glucose, insul
250 reported. In the panels B, C and D of the three Figures, 95% CI variations from time 0
251 were presented.
252
253 Discussion
254 The results of the present study indicate that the protein effects on both the thermogenic
255 the metabolic responses to meals. These changes may have practical implications for weight management
256 be considered when planning a healthy diet.
257
258 Energy expenditure and metabolic responses to meals

Our data show the meal-induced DIT after the morning meal was higher than that observed after dinner, while basal RMR were only slightly lower in the morning as compared with the evening as previously reported [7, 15]. Furthermore, our results indicate that FAFUAC values significantly increased after the evening meal rather than at morning.

A circadian pattern in the metabolic responses to nutrients could underlie the hypothesized DIT component. DIT consists of two components: an obligatory component, linked to the energy cost of absorption and metabolism of nutrients, and a facultative component, mediated mostly likely by the sympathetic nervous [23, 32-34]. A circadian rhythm in circulating norepinephrine has been found, with increased values [25] in the morning. Epinephrine increases metabolic rate, by increasing oxidation [20]. Other explanations for the evening-to-morning difference in thermic and macronutrient oxidation may be the slower gastric emptying, with efficiency of dietary carbohydrates absorption [35], and the reported decrements in sensory pleasantness throughout the day [36]. Possible contributors to the variations in such responses, with higher morning fractional oxidation, could be the circadian fluctuations in concentrations of GLP-1, a gut incretin peptide 1, the glucose-dependent insulinotropic polypeptide as well as the meal-induced glucagon responses, delayed after evening meals [2-3, 27-29]. Insulin resistance and determined increased thermic effect of glucose diminish insulin-mediated glucose uptake and metabolism by skeletal muscle [37] that may thermogenesis [38, 39]. Furthermore, sympathetically mediated thermogenesis under conditions of hyperinsulinemia/insulin resistance [23, 30] and the reported circadian variation in cate concentrations in such a way may contribute to the evening reductions in DIT. The reported reduced insulin sensitivity at evening, we found lower glucose delayed and larger increases in the concentrations of glucose (Figure 2 and Table S2, Supplementary Information).

A circadian control of FFA metabolism might have been overlooked in our study, as blood samples were collected only in the morning [31] and afternoon [2], and an increased activity of lipoprotein lipase [27]. The increased activity of clock target genes in adipocytes may impact on lipid breakdown and adipocyte mobilization from adipose tissue, thus acting on energy storage homeostasis and difficult to assess with our data if the higher increase in FFA AUC after the consequence of insulin resistance or rather the cause of the impaired effects of FFA on the reduction in glucose uptake in skeletal muscles and other impairment in insulin signaling and action [32,33].

Not the authors found a lower DIT after [21]. We observed a significant difference between morning and afternoon DIT, but not when the fasting period was shorter in the afternoon than in the morning and the energy contributions of the were not the same [9]. Interestingly, DIT was slightly, being energy balance (the difference between energy intake and expenditure) by the meal values of RQs and CHO oxidation significantly, in contrast with our results [9]. A study found a higher energy expenditure during enteral, but not oral, nutrition in patients hospitalized in a Neurologic unit on artificial therefore these results are difficult to compare with those in other healthy individuals. Similarly, we also found no difference in 24-h energy expenditure as measured in a respiratory chamber between normal (7:00 pm) or (10:30 pm) evening meal. Japanese measure of DIT was time-averaged, the experimental meals were not isocaloric and may not be consistent with our results. Postprandial glucose values were significantly increased [14]. Similarly, delaying the time identical meal-to-bed by 3 h did not change postprandial energy expenditure oxidation and glucose tolerance [12]

In our study performed under extreme conditions (an acute high-fat intake combined with prolonged fasting), which is known to exert a large energy expenditure [37], the thermic effect of the mixed meal we could have found lower values of DIT differences between the morning and evening. Nevertheless, the circadian variation in DIT might explain at least in part the individuality in variability in DIT and CHO and fat oxidation not by a circadian modulation but by external antecedents or in the method of measurement [17, 18]. The increase in morning-evening differences in DIT supports the view that energy expenditure was calculated in relation (Table 2).

Respiratory Quotients

RQ values generally range within 0.70 (100% lipid oxidation at 0.70, and 100% oxidation at 1.00). We found reduced CHO oxidation and RQ values and increased the evening, suggesting a shift of the metabolic pathways toward a higher utilization in the evening, supported by the data on the course of FFA levels after the evening meal (although starting from higher FFA values (Figure 3, panel A).

After the experimental infusion [23] glucose was similar, but a marked increase in CHO oxidation and a decrease in lipid oxidation occurred with. Other studies have found higher RQ in the morning [7, 9, 21, 38]. We significantly higher lipid oxidation CHO oxidation were described with mean values of 0.86 at 8:00 and 0.82 at 16:00 in subjects both after a short (3 days) and prolonged (18 days) fast [39]. It can be considered the time of the day in which an abrupt circadian shift takes place in the circadian rhythm glycogen synthesis [40]. Even if delta RQs were after slightly higher (Table 2), increased evening fat oxidation and FFA AUC sums suggest in line with the higher ratios that in resistance clock, a preferential use of lipid substrates in our sample

Clinical perspectives

Human studies have shown that adolescents consuming a greater proportion of the daily energy intake at evening is associated with an increased risk of obesity, hyperglycemia, liver steatosis, metabolic syndrome, alcoholic fatty liver disease and cardiovascular diseases [14, 21, 42, 43, 44, 45]. Circadian misalignment has adverse metabolic and cardiovascular consequences [46]. Examples of circumstances of this phenomenon are shift work and sleep deprivation, which have also been associated with an increased risk of obesity, metabolic syndrome, diabetes and cardiovascular diseases [31, 32, 47].

Timing of meals influences the success of weight loss treatments. Eating breakfast rather than early eating [48] or consuming more calories at lunch than at dinner [49] have been associated with greater weight loss and more successful long-term weight loss maintenance [47]. Therefore, dietary recommendations should not only include indications for the preferred consumption, besides advice on food choice and quantity.

Limitations

First of all, some issues need to be considered when trying to link results from short-term studies to long-term effects. We did not evaluate the energy expenditure during the three hours after the meal, which is more relevant [7], but other authors who recommended this measurement [31]. However, our experiment was consistent with studies showing energy expenditure increased after a meal and that DIT response to meals can be assessed sufficiently for the purpose of comparison across subjects [7, 45]. Furthermore, most of the differences in the metabolic patterns we found were small. We used the glucose equation to calculate EE from oxidation rates [25], which is a valid method [50], plasma glucose turnover is lower in lean than in obese subjects, but comparisons were performed

individuals under the same conditions [25], assuming exclusive glucose oxid
not introduce a major error.

Conclusions

Consuming a high-carbohydrate evening meal seems an unfavorable metabolically
unfavorable respect to the consumption of the same meal at morning. En
metabolism may be tightly linked to circadian rhythms; gaining further
useful to curb the current increasing rate of metabolic disorders.

Acknowledgments

The authors thank Professor Gianfranco Peaccetti for his critical reading of the
manuscript. This study was supported by a grant from the University and Res
Italy (60% 2014).

Conflicts of interest

The authors report no conflict of interest.

Supplementary information is available at journal's website.

References

1) Garaulet M, Gómez-Abellán P. Timing of food intake and obesity: a novel association. Physiol Behav. 2014;134:44-50.

2) Lindgren O, Mari A, Deacon CF, Carr RD, Winzell MS, Vikman J, Ahrén B. Differential islet and incretin hormone responses in morning versus afternoon after standardized meal in healthy men. J Clin Endocrinol Metab. 2009;94:2887-2892.

3) Van Cauter E, Desir D, Decoster C, Fery F, Balasse EG. Nocturnal decrease in glucose tolerance during constant glucose infusion. J Clin Endocrinol Metab. 1989;69:604-611.

4) Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc Natl Acad Sci USA. 2009;106:4453-4458.

5) Goo RH, Moore JG, Greenberg E, Alazraki HP. Circadian variation in gastric emptying of meals in humans. Gastroenterology. 1987;93:515-518.

6) Tsuchida Y, Hata S, Sone Y. Effects of a late supper on digestion and absorption of carbohydrates in the following morning. J Physiol Anthropol. 2014;33:21.

7) Romon M, Edme JL, Boulenguez C, Lescroart JL, Frimat P. Circadian variation of diet-induced thermogenesis. Am J Clin Nutr. 1993;57:476-480.

8) Ravussin E, Acheson KJ, Vernet O, Danforth E, Jéquier E. Evidence that insulin resistance is responsible for the decreased thermic effect of glucose and carbohydrate overfeeding in obesity. J Clin Invest. 1985;76:1268-1273.

9) Weststrate JA, Poortvliet EJ, Deurenberg P. Meal-induced thermogenesis in adolescents differing in postabsorptive resting metabolic rate. Am J Clin Nutr. 1990;51:908-914.

10) Sato M, Nakamura K, Ogata H, Miyashita M, Tanahashi T, Adachi Y, Kumano-go T, Akabayashi A. Effect of a late evening meal on diurnal variation of blood glucose and energy metabolism. Obes Res Clin Pract. 2011;5:e222-e228.

11. Leuck M, Levandovski R, Harb A, Quiles C, Hidalgo MP. Circadian oxygen consumption. J Parenter Enteral Nutr 2014;38:326-328.

12. Bandin C, Scheer FAJL, Garaulet M, Lopez-Minguez J, Madrid JA, Gomez-Abellan P, Garcia-Moreno H, Gonzalez-Abraldes I, Ordovas JM, Perez-Llamas F, Zamora S. Meal timing affects glucose tolerance, substrate oxidation and circadian-related variables: a randomized, crossover, pilot intervention study. Int J Obes (Lond) 2014. doi: 10.1038/ijo.2014.229. [Epub ahead of print]

13. Reed GW, Hill JO. Measuring the thermic effect of food. Am J Clin Nutr 1996;63:164-169.

14. Hibi M, Masumoto A, Naito Y, Kiuchi K, Yoshida H, Ishimoto Y, Hashimoto T, Tsuji T, Kawahara T. Nobiletin intake enhances peripheral circadian rhythms and prevents obesity in mice fed a high-fat diet. Am J Physiol Regul Integr Comp Physiol 2012;303:R1287-1295.

15. Westerterp KR. Diet induced thermogenesis. Nutr Metab (Lond) 2007;4:5.

16. Westerterp KR. Assessment of physical activity: a critical appraisal. Eur J Appl Physiol Occup Physiol 1999;80:539-549.

17. Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. J Chronic Dis 1978;31:741-755.

18. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol 1994;139:1197-1209.

19. Bo S, Ciccone G, Durazzo M, Gambino R, Fadda M, Massaia M, Pagano GF. Antioxidant treatment reducing resistin serum levels: a randomized controlled trial. Horm Res 2007;67:27-34.

20. Moreira da Rocha EE, Alves MG, Fernandes RA, Fonseca VM. Indirect calorimetry: methodology, instrumentation and clinical application. Curr Opin Clin Nutr Metab Care 2006;9:247-256.

21. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. Physiol 1949;109:1-9.

22. Ferrannini E. The theoretical basis of indirect calorimetry: a review. Metabolism 1988;37:287-301.

22) Allison DB, Paultre F, Maggio C, Mezzitis N, Pi-Sunyer FX. The use of areas under curves in research. Diabetes Care. 1995;18:1324-1329.

23) Acheson KJ, Ravussin E, Wahren J, Jéquier E. Thermic effect of glucose in man. Obligatory and facultative thermogenesis. J Clin Invest. 1984;74:1572-1580.

24) Astrup A, Simonsen L, Bülow J, Madsen J, Christensen NJ. Epinephrine mediates facultative carbohydrate-induced thermogenesis in human adipose tissue. Am J Physiol. 1985;249:E558-564.

25) Linsell CR, Lightman SL, Mullen PE, Brown MJ, Causon RC. Circadian rhythms of epinephrine and norepinephrine in man. J Clin Endocrinol Metab. 1985;60:1210-1215.

26) Müller MJ, Acheson KJ, Jéquier E, Schutz Y, Burger AG. Thermic effect of food and insulin: no role for endogenous insulin release. Metabolism. 1982;31:1158-1163.

27) Al-Naimi S, Hampton SM, Richard P, Tzung C, Morgan LM. Postprandial metabolic profiles of meals and snacks eaten during simulated night and day shift work. Chronobiol Int. 2004;21:933-947.

28) Van Cauter E, Shapiro ET, Tillil H, Polonsky KS. Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. Am J Physiol. 1992;262:E467-475.

29) Goetz F, Bishop J, Halberg F, Sothern RB, Brunning R, Sothern J, Mitchell H. Sex hormone patterns in relation to aging in the rat. Exp Aging Res. 1978;4:259-289.

30) Astrup A, Andersen T, Henriksen O, Christensen NJ, Bülow J, Madsen J. Evidence against a role for the sympathetic nervous system in the regulation of thermogenesis in skeletal muscle in man. Clin Sci (Lond). 1985;68:111-116.

31) Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. Proc Natl Acad Sci U S A. 2012;109:2625-2629.

32) Shostak A, Husse J, Oster H. Circadian regulation of adipose function. Adipocyte. 2013;2:196-202.

33) Huang W, Ramsey KM, Marcheva B, Bass J. Circadian rhythms, sle
2011;211-233.

34) Randle PJ. Regulatory interactions between lipids and carbohydra
35 years on). Diabetes Metab Rev 1998;14:263-283.

35) Roden M, Prince TB, Perseghin G, Petersen KF, Rothman DL, Clin
induced insulin resistance in overweight adolescents. J Clin Invest 1999;103:427-434.

36) Westerterp KR, Wilson SA, Rubbert A. Diet induced thermogenesis measured over 24 h in a respiration
chamber: effect of diet composition, food intake and body fatness in patients with anorexia nervosa. Br J Nutr 1992;68:321-332.

37) Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is increased 100% on a high-protein, low-
diet versus high-carbohydrate, low-fat diet in healthy, young women. J Am Coll Nutr 2002;21:55-61.

38) Farshchi HR, Taylor MA, Macdonald IA. Decreased thermic effect of food after an isocaloric high-fat meal
with a regular meal pattern in healthy lean women. Int J Obes Relat Metab Disord 2004;28:653-660.

39) Chwalibog A, Thorbek G. Energy expenditure and oxidation of carb
day and the thermochimica Acta 2002;394:241-252.

40) Sensi S, Capani F. Chronobiological aspects of diet, weight loss and body
regimen. Chronobiologia 1984;11:225-261.

41) Sollberger A. The control of circadian periodicity. Ann N Y Acad Sci 1964;117:554-564.

42) Bo S, De Carli L, Venco E, Fanzolato I, Manti D, Spada Vinciguerra M, Pagano G, Cavallo-Perin P,
overweight and obesity risk in adolescents. Results of the Italian Gastroenterology 2006;94:1057-
47.

43) Wang JB, Patterson RE, Ang A, Edmond JA, Shetty N, Arab L. Tim
associated with the risk of the metabolic syndrome. J Hum Nutr Diet 2012;25:255-262.

44. Bo S, Musso G, Biroli M, Fadda M, Fedele D, Gambino R, Gentile L, Durazzo M, Ghigo E, Cassader M, Cavallo-Perin P: Consuming more of daily calories at dinner predisposes to body fat gain and impaired metabolic profile over time: results of the prospective CALORIE-GRID study. Nutr Metab Cardiovasc Dis 2014, 24:1203-1209.

45. Cahill LE, Chiuve SE, Mekary RA, Jensen MK, Flint AJ, Hu FB, Rimm EB: Prospective study of breakfast eating and incident coronary heart disease in a cohort of male US health professionals. Circulation 2013, 128:337-343.

46. Gonnissen HK, Rutters F, Mazuy C, Martens EA, Adam TC, Westerterp-Plantenga MS: Effect of a phase advance and phase delay shift of 4 h in the sleep-wake cycle on hormonal appetite- and energy metabolism regulation in healthy lean men. Eur J Clin Nutr 2013, 67:1176-1182.

47. Garaulet M, Gómez-Abellán P, Alburquerque-Béjar JJ, Lee YC, Ordovás JM, Scheer FA: Timing of food intake predicts weight loss effectiveness. Int J Obes (Lond) 2013, 37:604-611.

48. Jakubowicz D, Barnea M, Wainstein J, Froy O: High caloric intake at breakfast vs. dinner differentially influences weight loss of overweight and obese women. Obesity (Silver Spring) 2013, 21:2504-2512.

49. Segal KR, Edano A, Abalos A, Albu J, Blando W, Gutin B, Pi-Sunyer FX: Thomas MB. Thermic effect of a meal over 3 and 6 h. Metabolism 1990, 39:998-1002.

Figure Legends

Figure 1

The arrows indicate the times at which samples were drawn. During the visit, p
submitted to anthropometric measurements and was peripherally cannulated. Th
to the time before the first calorimetric examination, which was performed 1 h
and before the meal. The times 30, 60, 90, 120, 150 and 180 were refe
the beginning of the meal.

The same time schedule was adopted in the case of the evening meal (at
8:00 pm).

Figure 2

Mean glucose values at the different time points (panel A). Variation of glucose from time 0
changes from the value at time 0 (black dot), evening mean). The grey dot
whiskers indicate the 95% CIs (panel

Figure 3

Mean insulin values at the different time points (panel A). Variation of
changes from the value at time 0 (black dot), evening mean). The changes fr
whiskers indicate the 95% CIs (panels A and B).

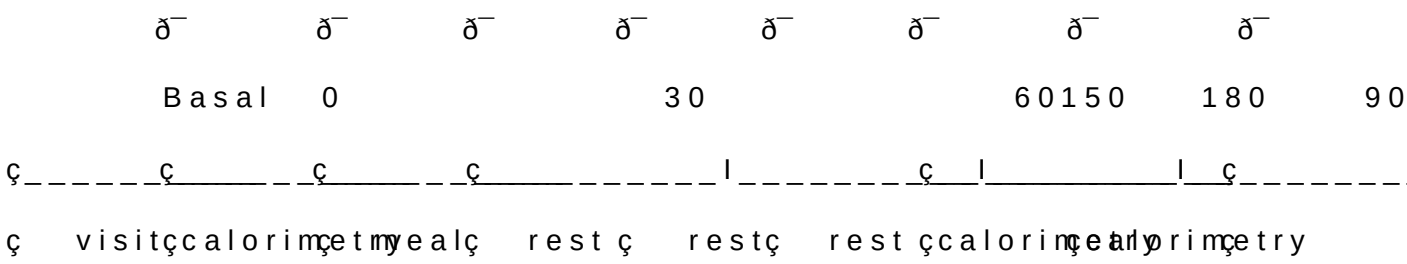
Figure 4

Mean FFA values at the different time points (panel A). Variation of FF
from the value at time 0 (black dot), evening mean (grey dot). The whiskers
indicate the 95% CIs (panels A and B).

Figure 5

Mean triglyceride values at the different time points (panel A). Variation of triglycerides from
mean changes from the value at time 0 (black dot), evening mean chan
The whiskers indicate the 95% CIs (panels A and B).

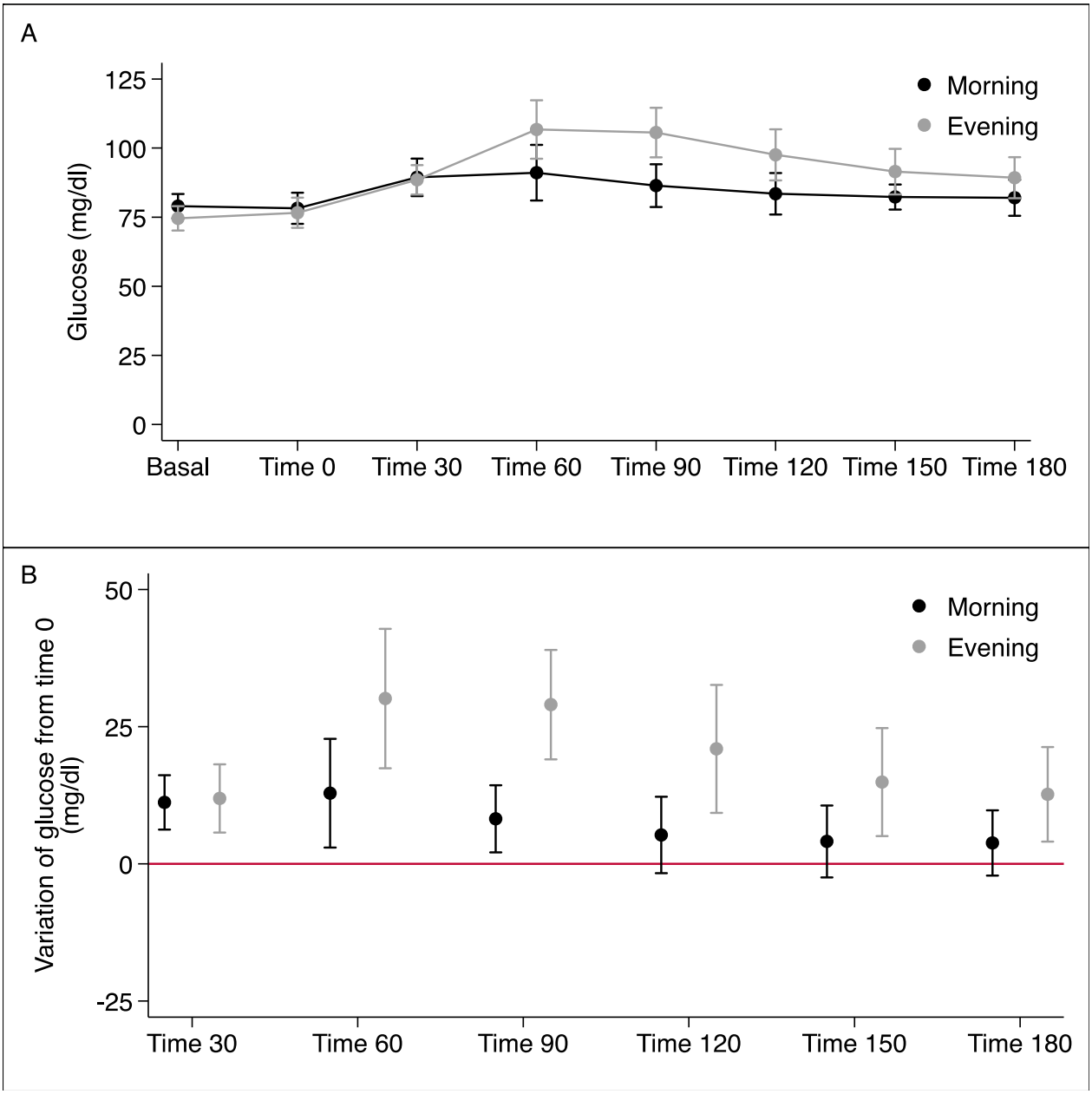
Figure 1. Time schedule of the study



The arrows indicate the time when the blood samples were drawn. During the time the subject was submitted to anthropometric measurements, blood was collected peripherally contralateral to the one used for the blood samples. The time 0 corresponds to the time before the first calorimetric examination was performed. The time 30 corresponds to the time after the first calorimetric examination and before the meal. The times 30, 60, 90, 120, 150 and 180 were measured in minutes from the beginning of the meal.

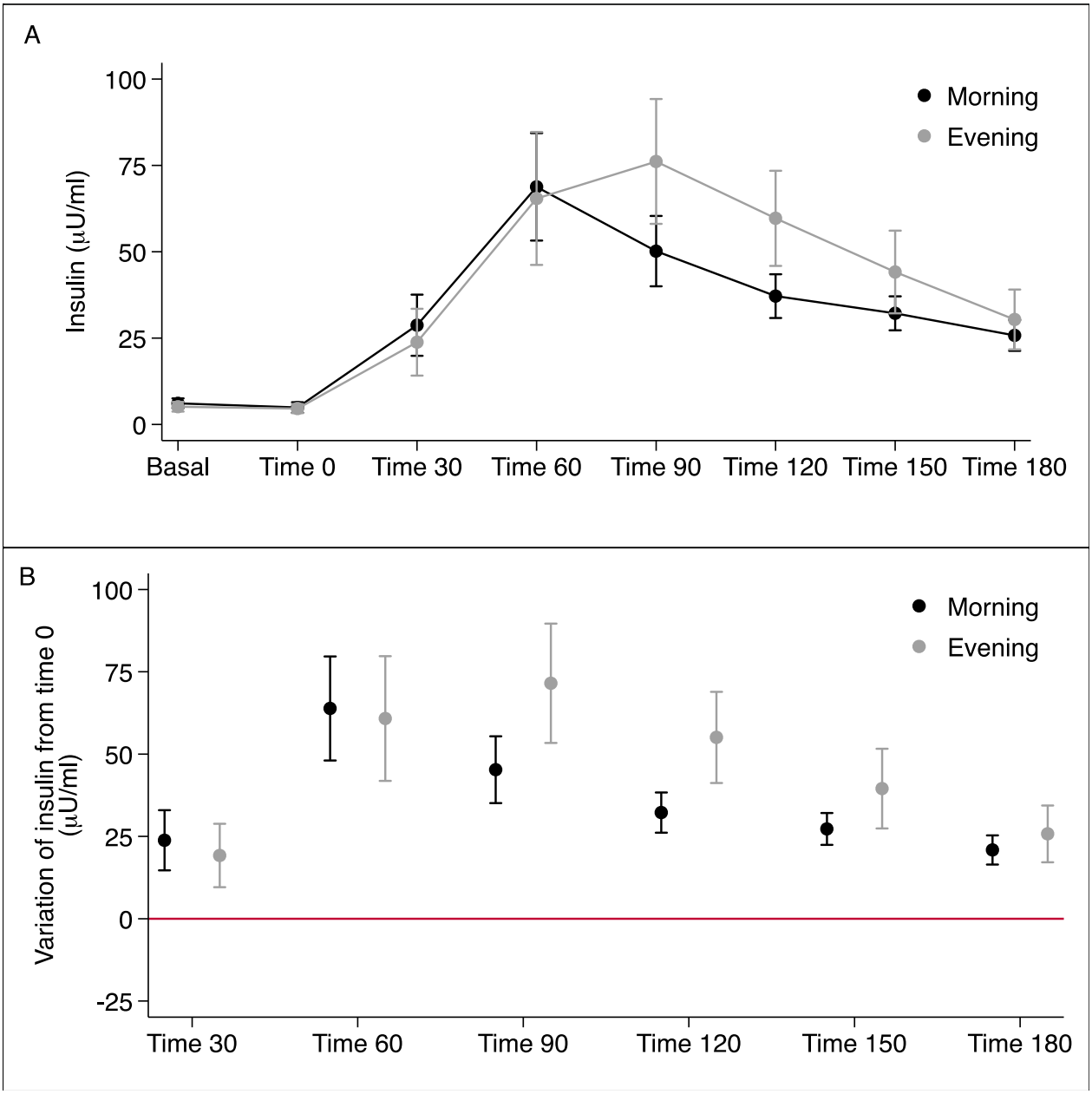
The same time schedule was adopted in the case of the morning meal (8:00 am).

Figure 2. Mean glucose values at the different time points (panel A) and variation o
(panel B)



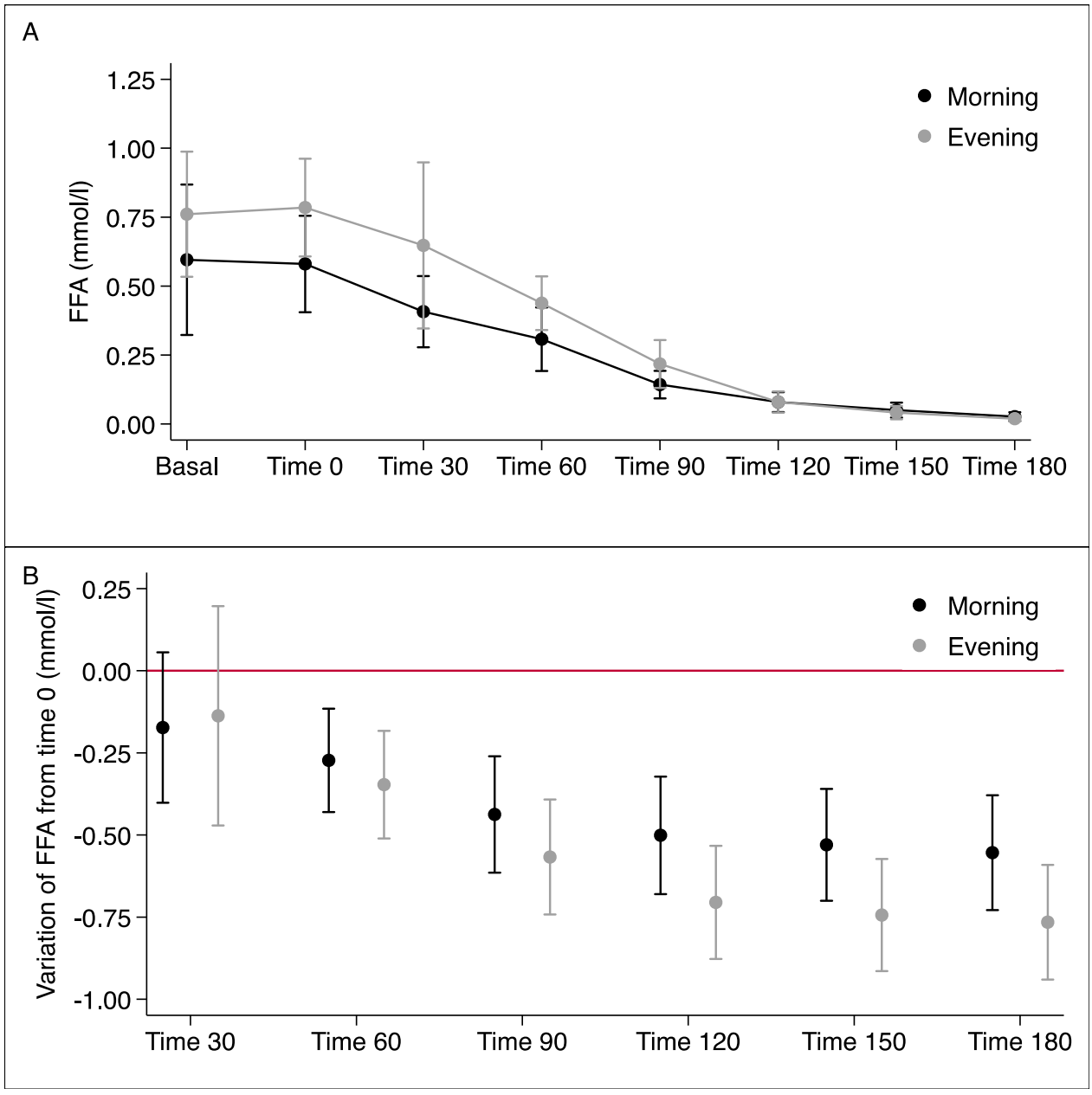
Mean glucose values at the different time points (panel A). Variation of glucose from time 0: mo
changes from the value at time 0 (black dot), evaluated at each time point (panel B). The grey dot
whiskers indicate the 95% CIs (panel

Figure 3. Mean insulin values at different time points (panel A) and variation from time 0 (panel B)



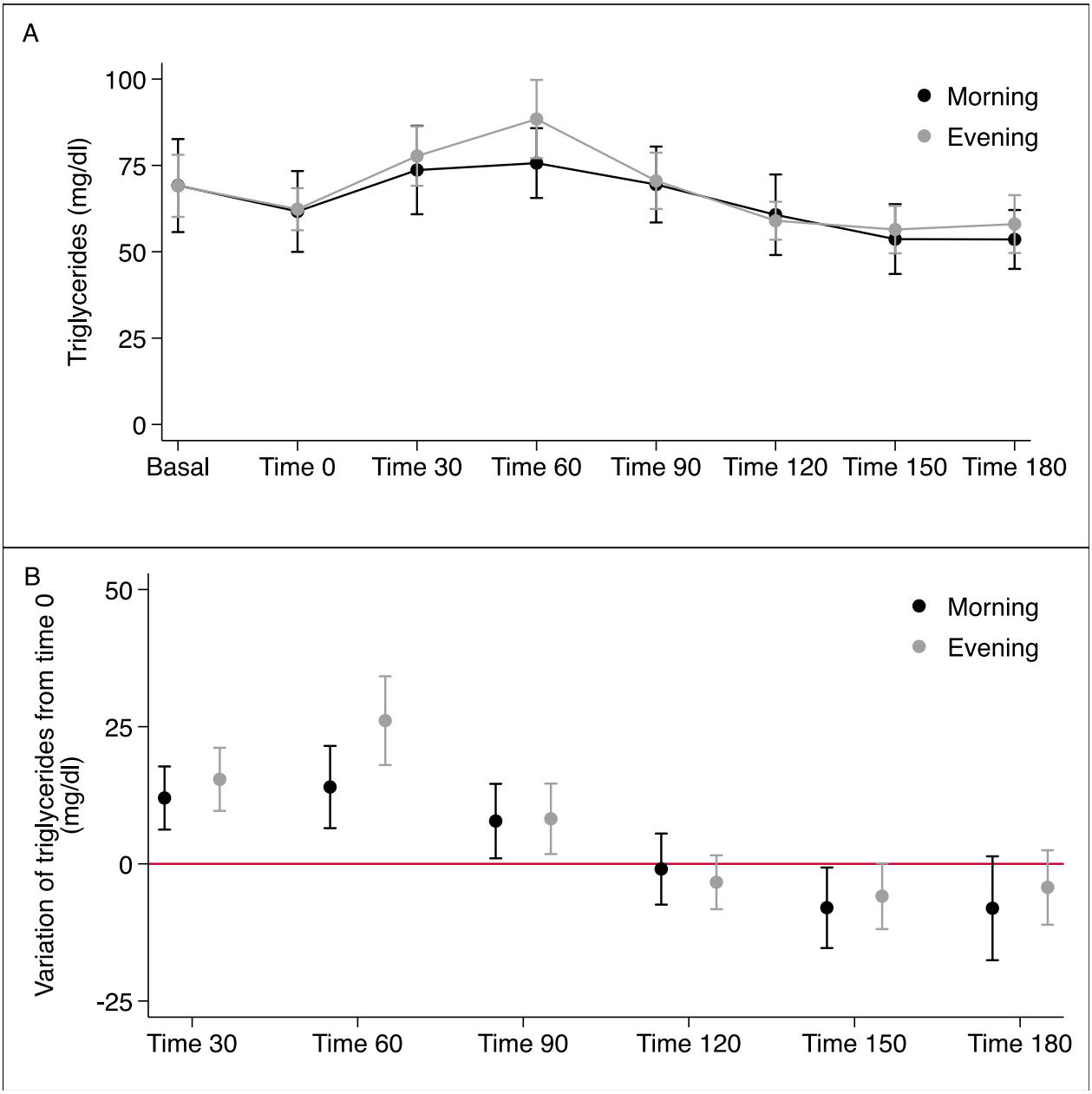
Mean insulin values at the different time points (panel A). Variations of insulin from time 0 values (from the value at time 0), at the same time points (panel B). Changes from the value at time 0 (black dot), with 95% BCIs (panel C). The grey dot whiskers indicate the 95% BCIs.

Figure 4. Mean and variation of FFA values at different time points (panel A) and variation of F



Mean FFA values at the different time points (panel A). Variation of FFA from time 0: morning and evening (black and grey, respectively) changes from the value at time 0 (panel B). The whiskers indicate the 95% CI (panel

Figure 5. Mean triglyceride values at different time points (panel A) and variation from time 0 (panel B)



Mean triglyceride values at the different time points (panel A). Variation of triglycerides from mean change from the value at time 0 (black dot) or the value at time 0 (grey dot) (panel B). The whiskers indicate the 95% CIs (panel A and B).

Table 2 Calorimetric variables before and after morning and evening mea

	Morning		Evening		p-value
Number	20		20		
Fasting RMR (kcal)	1588.	[1464.9;1	1518.	[1407.7;1	0.098
After meal RMR (kcal)	1916.	[1791.5;2	1755.	[1648.0;1	<0.00
DIT (kcal)	327.5	[279.0;37	237.0	[195.1;2	0.003
Fasting [1] RMR (kcal/kg FFI	30.2	[27.8;31	29.1	[27.0;31	0.180
After meal [1] RMR (kcal/kg F	36.7	[34.6;38	33.7	[31.4;36	<0.00
DIT [1] (kcal/kg FFM)	6.46	[5.16;7.	4.62	[3.55;5.	0.003
Fasting RQ	0.87	[0.85;0.	0.80	[0.78;0.	<0.00
After meal RQ	0.90	[0.89;0.	0.85	[0.82;0.	0.002
RQ Difference	0.03	[0.01;0.	0.05	[0.02;0.	0.055
Fasting CHO oxidat	0.13	[0.10;0.	0.05	[0.02;0.	<0.00
After meal CHO oxidat	0.20	[0.18;0.	0.12	[0.08;0.	<0.00
CHO oxidation diffe	0.07	[0.05;0.	0.08	[0.05;0.	0.856
Fasting fat oxidatio	0.01	[0.01;0.	0.04	[0.03;0.	<0.00
After meal fat oxidation (g	0.01	[0.00;0.	0.03	[0.02;0.	0.006
Fat oxidation differ	-0.01	[-0.01;0.	-0.01	[-0.03;0.	0.116

Mean [95% CI]; p calculated by t-tests for paired data

RMR = Resting Metabolic Rate; DIT = Diet-Induced Thermogenesis; RQ = Respiratory Quotient; CHO carbohydrates

[1] energy expenditure corrected in relation to fat free mass

Table 2. Estimates of morning effect adjusted for by Generalized Linear Mixed Models (GLMs)

	Effects	95% CI	p-value
RMR[1] (kcal)	90.5	[40.4, 140.6]	<0.001
RMR[1,2] (kcal/kg FFM)	1.84	[0.81, 2.87]	<0.001
RQ[3]	-0.02	[-0.04, 0.00]	0.035
CHO oxidation[3] (g/min)	0.00	[-0.02, 0.02]	0.848
Fat oxidation[3] (g/min)	0.01	[-0.00, 0.02]	0.089
Glucose[4] (AUC, mM*h)	-1800.1	[-2564, -1036.0]	<0.001
Log insulin[4,5] (AUC, mU/ml*h)	-0.19	[-0.30, -0.07]	0.001
Log insulin[4,6] AUC	0.83	[0.74, 0.93]	0.001
FFA[4] (AUC, mM*h)	-16.1	[-30.2, -2.09]	0.024
Log triglycerides (small AUC)[4,5]	-0.08	[-0.21, 0.05]	0.230
Log triglycerides AUC[4,6]	0.92	[0.81, 1.05]	0.230

RMR = Resting Metabolic Rate; RQ = Respiratory Quotient; CHO = carbohydrates; AUC = area under the curve; FFA = free fatty acids

[1] morning-delta Diet-Induced Thermogenesis (DIT); DIT

[2] RMR calculated in relation to fat free mass

[3] Morning delta minus evening delta

[4] Morning AUC vs. evening AUC

[5] Estimated effects expressed as mean difference in log

[6] Estimated effects expressed as ratio.